Rx Only

cobas® SARS-CoV-2

Qualitative assay for use on the cobas® 6800/8800 Systems

For use under the Emergency Use Authorization (EUA) only

For in vitro diagnostic use

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cobas® SARS-CoV-2	P/N: 09175431190
cobas® SARS-CoV-2 Control Kit	P/N: 09175440190
cobas® 6800/8800 Buffer Negative Control Kit	P/N: 07002238190

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Intended use

cobas® SARS-CoV-2 for use on the **cobas**® 6800/8800 Systems is a real-time RT-PCR test intended for the qualitative detection of nucleic acids from SARS-CoV-2 in nasopharyngeal and oropharyngeal swab samples from patients who meet COVID-19 clinical and/or epidemiological criteria. **cobas**® SARS-CoV-2 is for use only under Emergency Use Authorization (EUA) in U.S. laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate complexity tests, and in U.S. laboratories certified under CLIA to perform high complexity tests, by clinical laboratory personnel who have received specific training on the use of the **cobas**® 6800/8800 Systems.

Results are for the detection of SARS-CoV-2 RNA that are detectable in nasopharyngeal and oropharyngeal swab samples during infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

cobas® SARS-CoV-2 is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. **cobas**® SARS-CoV-2 is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and explanation of the test

Explanation of the test

cobas® SARS-CoV-2 is a qualitative test on the **cobas**® 6800 System and **cobas**® 8800 System for the detection of the 2019 novel coronavirus (SARS-CoV-2) RNA in nasopharyngeal and oropharyngeal swab samples collected in Copan Universal Transport Medium System (UTM-RT) or BD™ Universal Viral Transport System (UVT). The RNA Internal Control, used to monitor the entire sample preparation and PCR amplification process, is introduced into each specimen during sample processing. In addition, the test utilizes external controls (low titer positive control and a negative control).

Principles of the procedure

cobas® SARS-CoV-2 is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**® 6800/8800 software, which assigns test results for all tests. Results can be reviewed directly on the system screen, and printed as a report.

Nucleic acid from patient samples and added internal control RNA (RNA IC) molecules are simultaneously extracted. Nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors, are removed with subsequent wash steps and

purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature. External controls (positive and negative) are processed in the same way with each **cobas**® SARS-CoV-2 run.

Selective amplification of target nucleic acid from the sample is achieved by the use of target-specific forward and reverse primers for ORF1/a non-structural region that is unique to SARS-CoV-2. Additionally, a conserved region in the structural protein envelope E-gene were chosen for pan-Sarbecovirus detection. The pan-Sarbecovirus detection sets will also detect SARS-CoV-2 virus.

Selective amplification of RNA Internal Control is achieved by the use of non-competitive sequence specific forward and reverse primers which have no homology with the coronavirus genome. A thermostable DNA polymerase enzyme is used for amplification.

The **cobas**® SARS-CoV-2 master mix contains detection probes which are specific for the coronavirus type SARS-CoV-2, members of the Sarbecovirus subgenus, and the RNA Internal Control nucleic acid. The coronavirus and RNA Internal Control detection probes are each labeled with unique fluorescent dyes that act as a reporter. Each probe also has a second dye which acts as a quencher. When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Each reporter dye is measured at defined wavelengths, which enables simultaneous detection and discrimination of the amplified coronavirus target and the RNA Internal Control. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicons from previous PCR runs are destroyed by the AmpErase enzyme [uracil-N-glycosylase], which is included in the PCR mix, when heated in the first thermal cycling step. However, newly formed amplicons are not destroyed since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

Reagents and materials

The materials provided for **cobas**® SARS-CoV-2 can be found in Table 1 and Table 2. Materials required, but not provided can be found in Table 3, Table 4, Table 7, and Table 8.

Refer to the **Reagents and materials** section and **Precautions and handling requirements** section for the hazard information for the product.

cobas® SARS-CoV-2 reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 4.

Table 1 cobas® SARS-CoV-2

cobas® SARS-CoV-2 Store at 2-8°C 192 test cassette (P/N 09175431190)		
Kit components	**Reagent ingredients**	**Quantity per kit 192 tests**
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin. May produce an allergic reaction.	22.3 mL
RNA Internal Control (RNA IC)	Tris buffer, <0.05% EDTA, <0.001% non-Sarbecovirus related armored RNA construct containing primer and probe specific primer sequence regions (non-infectious RNA in MS2 bacteriophage), <0.1% sodium azide	21.2 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	21.2 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 mL
SARS-CoV-2 Master Mix Reagent 2 (SARS-CoV-2 MMX-R2)	Tricine buffer, potassium acetate, < 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% upstream and downstream SARS-CoV-2 and Sarbecovirus primers, < 0.01% Internal Control forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for SARS-CoV-2, Sarbecovirus, and the RNA Internal Control, < 0.01% oligonucleotide aptamer, < 0.1% Z05D DNA polymerase, < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	9.7 mL

Table 2 cobas® SARS-CoV-2 Control Kit

cobas® SARS-CoV-2 Control Kit Store at 2–8°C (P/N 09175440190)		
Kit components	**Reagent ingredients**	**Quantity per kit**
SARS-CoV-2 Positive Control **(SARS-CoV-2 (+)C)**	Tris buffer, < 0.05% Sodium azide, < 0.005% EDTA, < 0.003% Poly rA, < 0.01% Non-infectious plasmid DNA (microbial) containing SARS-CoV-2 sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing pan-Sarbecovirus 1 sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing pan-Sarbecovirus sequence	16 mL (16 x 1 mL)

Table 3 cobas® Buffer Negative Control Kit

cobas® Buffer Negative Control Kit Store at 2-8°C (P/N 07002238190)		
Kit components	**Reagent ingredients**	**Quantity per kit**
cobas® Buffer Negative Control **(BUF (-) C)**	Tris buffer, < 0.1% sodium azide, EDTA, < 0.002% Poly rA RNA (synthetic)	16 mL (16 x 1 mL)

cobas omni reagents for sample preparation

Table 4 cobas omni reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	43% (w/w) guanidine thiocyanate***, 5% (w/v) polydocanol***, 2% (w/v) dithiothreitol***, dihydro sodium citrate	4 x 875 mL	  **DANGER** H302 + H332: Harmful if swallowed or if inhaled. H314: Causes severe burns and eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/fume/gas/mist/vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* These reagents are not included in the **cobas**® SARS-CoV-2 test kit. See listing of additional materials required (Table 7).

** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance

Reagent storage and handling requirements

Reagents shall be stored and will be handled as specified in Table 5 and Table 6.

When reagents are not loaded on the **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

Table 5 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® SARS-CoV-2 -192	2–8°C
cobas® SARS-CoV-2 Control Kit	2–8°C
cobas® Buffer Negative Control Kit	2–8°C
cobas omni Lysis Reagent	2–8°C
cobas omni MGP Reagent	2–8°C
cobas omni Specimen Diluent	2–8°C
cobas omni Wash Reagent	15–30°C

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas**® 6800/8800 Systems allow reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 6 Reagent expiry conditions enforced by the **cobas**® 6800/8800 Systems

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® SARS-CoV-2 – 192	Date not passed†	90 days from first usage*,†	Max 40 runs†	Max 40 hours†
cobas® SARS-CoV-2 Control Kit	Date not passed†	Not applicable[a]	Not applicable	Max 8 hours†
cobas® Buffer Negative Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 10 hours
cobas omni Lysis Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable

[a]Single use reagents

*Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.

†The performance has not been established for suggested use cycles and time, but is based on similar reagents used on the same system.

Additional materials required

Table 7 Materials and consumables for use on **cobas**® 6800/8800 Systems

Material	P/N
cobas omni Processing Plate	05534917001
cobas omni Amplification Plate	05534941001
cobas omni Pipette Tips	05534925001
cobas omni Liquid Waste Container	07094388001
cobas omni Lysis Reagent	06997538190
cobas omni MGP Reagent	06997546190
cobas omni Specimen Diluent	06997511190
cobas omni Wash Reagent	06997503190
Solid Waste Bag	07435967001
Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer	07435967001 and 07094361001 or 08030073001 and 08387281001
Solid Waste Container	07094361001
cobas omni Secondary Tubes 13x75 (optional)	06438776001

Instrumentation and software required

The **cobas**® 6800/8800 software and **cobas**® SARS-CoV-2 analysis package must be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 8 Instrumentation

Equipment	P/N
cobas® 6800 System (Moveable Platform)	05524245001 and 06379672001
cobas® 6800 System (Fixed Platform)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001
Instrument Gateway	06349595001

For additional information, please refer to the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide.

Note: Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For *in vitro* diagnostic use under Emergency Use Authorization only.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[1,2] Only personnel proficient in handling infectious materials and the use of **cobas**® SARS-CoV-2 and **cobas**® 6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- The use of sterile disposable pipettes and nuclease-free pipette tips is recommended. Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- **cobas**® SARS-CoV-2 test kit, **cobas**® SARS-CoV-2 Control kit, **cobas**® Buffer Negative Control kit, **cobas omni** MGP Reagent, and **cobas omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® SARS-CoV-2 kits, **cobas**® SARS-CoV-2 Control kit, **cobas**® Buffer Negative Control kit and **cobas omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Samples

- Follow manufacturer's instructions for collection, transport and storage of samples in Copan UTM-RT System (UTM-RT) or BD™ Universal Viral Transport System (UVT).
- Sample stability when using **cobas**® SARS-CoV-2 has not been established for suggested temperatures and time, but is based on viability data from testing similar viruses in the UTM-RT or UVT Systems as stated in Copan UTM-RT System Instructions For Use and shown below:
    - After collection, the specimen should be stored at 2-25 °C and processed within 48 hours.
    - If delivery and processing exceed 48 hours, specimens should be transported in dry ice and once in laboratory frozen at -70 °C or colder.

Instructions for use

Procedural notes

- Do not use **cobas**® SARS-CoV-2 reagents, **cobas**® SARS-CoV-2 Control Kit, **cobas**® Buffer Negative Control Kit, or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide for proper maintenance of instruments.

Running cobas® SARS-CoV-2

cobas® SARS-CoV-2 can be run with a minimum required sample volume of 0.6 mL.

Always use caution when transferring specimens from primary containers to secondary tube.

Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens.

Always use a new pipette tip for each specimen.

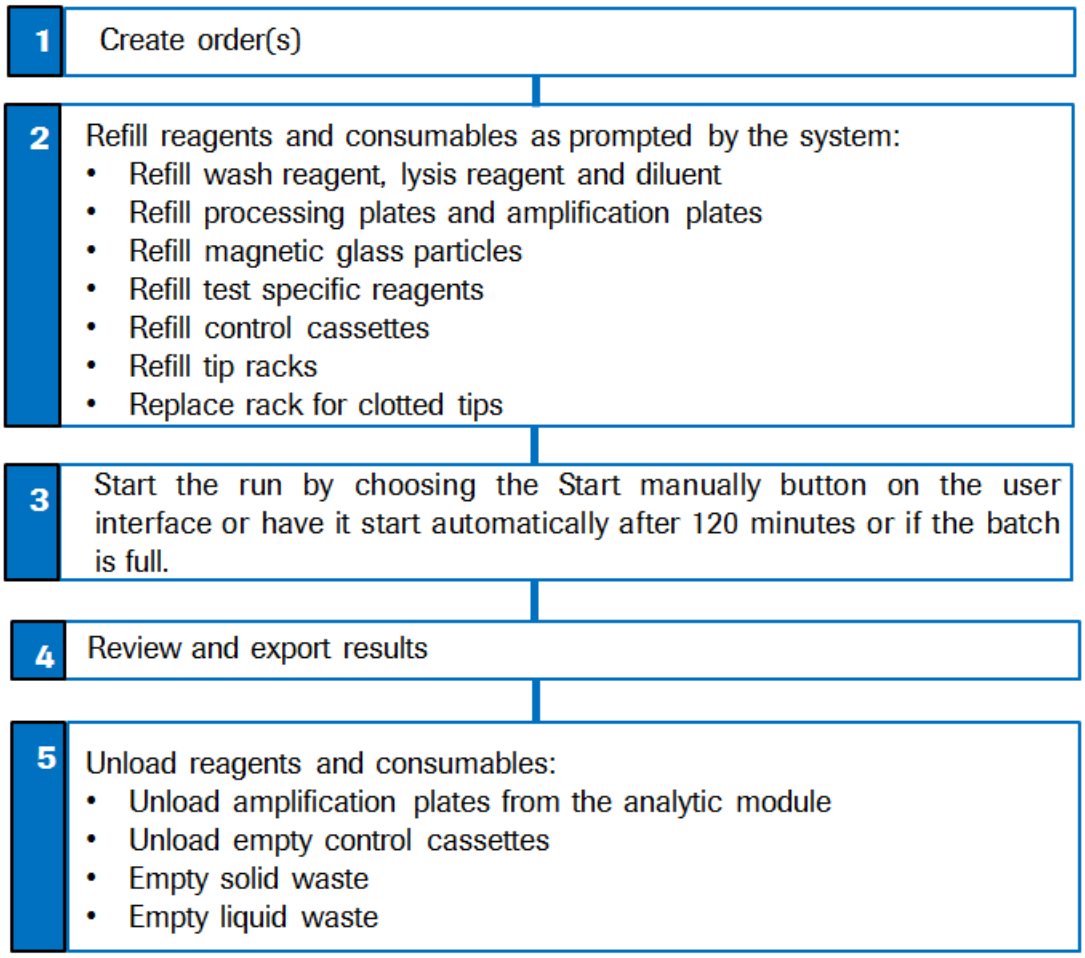
Ensure samples are equilibrated to room temperature prior to transfer into a cobas omni Secondary Tube.

Follow the steps below to transfer patient sample from a UTM-RT or UVT tube into a **cobas omni** Secondary Tube:

- Unscrew the primary sample tube cap.
- Lift the cap and any attached swab to allow a pipette to be inserted into the sample tube. Avoid lifting the swab completely out of the sample tube.
- Transfer 0.6 mL into the prepared barcoded secondary tube.
- Transfer secondary tube to a rack. Close the primary sample tube cap.

The test procedure is described in detail in the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide. Figure 1 below summarizes the procedure.

Figure 1 **cobas**® SARS-CoV-2 procedure



Results

The **cobas**® 6800/8800 Systems automatically detects the SARS-CoV-2 , for each individually processed sample and control, displaying individual target results for samples as well as test validity and overall results for controls.

Quality control and validity of results

- One **cobas**® Buffer Negative Control [(-) Ctrl] and one [SARS-CoV-2 (+)C] are processed with each batch.
- In the **cobas**® 6800/8800 software and/or report, check for flags and their associated results to ensure the batch validity.
- All flags are described in the **cobas**® 6800/8800 Systems User Guide.
- The batch is valid if no flags appear for any controls. If the batch is invalid, repeat testing of the entire batch.

Validation of results is performed automatically by the **cobas**® 6800/8800 software based on negative and positive control performance.

Interpretation of results

cobas® SARS-CoV-2 for System Software v1.2

Display examples for **cobas**® SARS-CoV-2 for System Software v1.2 or higher are shown in Figure 2.

Figure 2 Example of **cobas**® SARS-CoV-2 results display for System Software v1.2

Test	Sample ID	Valid*	Flags	Sample type	Overall result*	Target 1	Target 2
SARS-CoV-2 400 µL	Swab_01	Yes		Swab	**Negative**	Negative	Negative
SARS-CoV-2 400 µL	Swab _C1	No	Y40T	Swab	**Invalid**	Invalid	Invalid
SARS-CoV-2 400 µL	Swab _B1	Yes		Swab	**Reactive**	Negative	Positive
SARS-CoV-2 400 µL	Swab _B2	Yes		Swab	**Positive**	Positive	Positive
SARS-CoV-2 400 µL	Swab _D1	Yes		Swab	**Negative**	Negative	Negative
SARS-CoV-2 400 µL	Swab _A6	Yes		Swab	**Reactive**	Positive	Negative
SARS-CoV-2 400 µL	Swab _E1	No	C01H2	Swab	**Invalid**	Positive	Invalid
SARS-CoV-2 400 µL	Swab _A2	No	C01H1	Swab	**Invalid**	Invalid	Positive
SARS-CoV-2	C161420284090428828404	Yes		(-) Ctrl	**Valid**	Valid	Valid
SARS-CoV-2	C16142028409300958026 4	Yes		SARS-CoV-2 (+) C	**Valid**	Valid	Valid

* The "Valid" and "Overall Result" columns are not applicable to sample results for the **cobas**® SARS-CoV-2. Values reported in these columns are not applicable and do not impact the validity of results reported within individual Target Result columns. Refer to Table 9, **cobas**® SARS-CoV-2 results interpretation, for specific instructions on test results interpretation.

cobas® SARS-CoV-2 for System Software v1.3 or higher

Display examples for **cobas**® SARS-CoV-2 for System Software v1.3 or higher are shown in Figure 3.

Figure 3 Example of **cobas**® SARS-CoV-2 results display for System Software v1.3 or higher

Test	Sample ID	Valid*	Flags	Sample type	Overall result*	Target 1	Target 2
SARS-CoV-2 400 µL	Swab_01	NA		Swab	**NA**	Negative	Negative
SARS-CoV-2 400 µL	Swab _C1	NA	Y40T	Swab	**NA**	Invalid	Invalid
SARS-CoV-2 400 µL	Swab _B1	NA		Swab	**NA**	Negative	Positive
SARS-CoV-2 400 µL	Swab _B2	NA		Swab	**NA**	Positive	Positive
SARS-CoV-2 400 µL	Swab _D1	NA		Swab	**NA**	Negative	Negative
SARS-CoV-2 400 µL	Swab _A6	NA		Swab	**NA**	Positive	Negative
SARS-CoV-2 400 µL	Swab _E1	NA	C01H2	Swab	**NA**	Positive	Invalid
SARS-CoV-2 400 µL	Swab _A2	NA	C01H1	Swab	**NA**	Invalid	Positive
SARS-CoV-2	C161420284090428828404	Yes		(-) Ctrl	**Valid**	Valid	Valid
SARS-CoV-2	C16142028409300958026 4	Yes		SARS-CoV-2 (+) C	**Valid**	Valid	Valid

* The "Valid" and "Overall Result" columns are not applicable to sample results for the **cobas**® SARS-CoV-2. Values reported in these columns are not applicable and do not impact the validity of results reported within individual Target Result columns. Refer to Table 9, **cobas**® SARS-CoV-2 results interpretation, for specific instructions on test results interpretation.

Interpretation of results

The following result interpretation applies to both **cobas**® 6800/8800 software version 1.2 and **cobas**® 6800/8800 software version 1.3 and higher.

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.
- **The "Valid" and "Overall Result" columns are not applicable to sample results for the cobas® SARS-CoV-2. Values reported in these columns are not applicable and do not impact the validity of results reported within individual Target Result columns.**
- Invalid results for one or more target combinations are possible and are reported out specifically for each channel.
- Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.

Results and their corresponding interpretation for detecting SARS-CoV-2 are shown below (Table 9).

Table 9 **cobas**® SARS-CoV-2 results interpretation

Target 1	Target 2	Interpretation
Positive	**Positive**	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
Positive	**Negative**	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected. A positive Target 1 result and a negative Target 2 result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the Target 2, target region, or 3) other factors.
Negative	**Positive**	All Target Results were valid. Result for SARS-CoV-2 RNA is Presumptive Positive. A negative Target 1 result and a positive Target 2 result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the Target 1 target region in the oligo binding sites, or 3) infection with some other Sarbecovirus (e.g., SARS-CoV or some other Sarbecovirus previously unknown to infect humans), or 4) other factors. Sample should be retested. For samples with a repeated Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
Negative	**Negative**	All Target Results were valid. Result for SARS-CoV-2 RNA is Not Detected.
Positive	**Invalid**	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
Invalid	**Positive**	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Presumptive Positive. Sample should be retested. For samples with a repeated Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
Negative	**Invalid**	Not all Target Results were valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	**Negative**	Not all Target Results were valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	**Invalid**	All Target Results were invalid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.

Procedural limitations

- **cobas**® SARS-CoV-2 has been evaluated only for use in combination with the **cobas**® SARS-CoV-2 Control Kit, **cobas**® Buffer Negative Control Kit, **cobas omni** MGP Reagent, **cobas omni** Lysis Reagent, **cobas omni** Specimen Diluent, and **cobas omni** Wash Reagent for use on the **cobas**® 6800/8800 Systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- This test is intended to be used for the detection of SARS-CoV-2 RNA in nasopharyngeal and oropharyngeal swab samples collected in a Copan UTM-RT System (UTM-RT) or BD™ Universal Viral Transport System (UVT). Testing of other sample types with **cobas**® SARS-CoV-2 may result in inaccurate results.
- Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection.
- As with any molecular test, mutations within the target regions of **cobas**® SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies. Users should follow their own specific policies/procedures.
- False negative or invalid results may occur due to interference. The Internal Control is included in **cobas**® SARS-CoV-2 to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.
- The addition of AmpErase enzyme into the **cobas**® SARS-CoV-2 Master Mix reagent enables selective amplification of target RNA; however, good laboratory practices and careful adherence to the procedures specified in this Instructions For Use document are necessary to avoid contamination of reagents.

Non-clinical performance evaluation

Key performance characteristics

Analytical sensitivity

Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all (true positive) replicates test positive.

To determine the LoD, a cultured virus of an isolate from a US patient (USA-WA1/2020, catalog number NR-52281, lot number 70033175, 2.8E+05 $TCID_{50}$/mL^[1]) was serially diluted in simulated clinical matrix. A total of 7 concentration levels, with 3-fold serial dilutions between the levels, were tested with a total of 21 replicates per concentration, with an additional 10 replicates of a blank sample (i.e, simulated clinical matrix).

As shown in Table 10, the concentration level with observed hit rates greater than or equal to 95% were 0.009 and 0.003 $TCID_{50}$/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively. As shown in Table 11, the Probit predicted 95% hit rates were 0.007 and 0.004 $TCID_{50}$/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively.

Table 10 LoD determination using USA-WA1/2020 strain

Strain	Concentration [$TCID_{50}$/mL]	Total valid results	Hit rate [%]^		Mean Ct*	
			Target 1	Target 2	Target 1	Target 2
USA-WA1/2020 (stock concentration 2.8E+05 $TCID_{50}$/mL)	0.084	21	100	100	31.0	33.0
	0.028	21	100	100	31.8	34.1
	0.009	21	100	100	32.7	35.2
	0.003	21	38.1	100	33.5	36.4
	0.001	21	0	52.4	n/a	37.9
	0.0003	21	0	14.3	n/a	37.2
	0.0001	21	0	9.5	n/a	38.5
	0 (blank)	10	0	0	n/a	n/a

^ All replicates where Target 1 was positive were also positive for Target 2.

* Calculations only include positive results.

Table 11 Probit predicted 95% hit rates using USA-WA1/2020 strain

Strain	Probit Predicted 95% Hit Rate [$TCID_{50}$/mL]	
	Target 1	Target 2
USA-WA1/2020 (stock concentration 2.8E+05 $TCID_{50}$/mL)	0.007 (95% CI: 0.005 – 0.036)	0.004 (95% CI: 0.002 – 0.009)

[1] The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-WA1/2020, NR-52281.

Reactivity/inclusivity

In silico analysis concluded that **cobas**® SARS-CoV-2 will detect all analyzed SARS-CoV-2 sequences in NCBI and in GISAID databases.

cobas® SARS-CoV-2 had 100% match to all but one sequence for Target 1 (NCBI (n=79); GISAID (n=366)). For the one sequence, a single nucleotide mismatch was found that maps to the 5'-end of the reverse primer, with no predicted impact on the assay performance.

cobas® SARS-CoV-2 had 100% match to all but three sequences for Target 2 (NCBI (n=81); GISAID (n-364)). For one sequence, a single nucleotide mismatch was found close to the 3'-end of the probe binding region. For a second sequence, a single mismatch was found at the 3'-end of the forward primer binding region. For a third sequence, a single mismatch was found at the 3'-end of the reverse primer binding region. None of these single base mismatches are predicted to impact the performance.

Cross-reactivity

In silico analysis

The *in silico* analysis for possible cross-reactions with all the organisms listed in Table 13 was conducted by mapping primers in **cobas**® SARS-CoV-2 individually to the sequences downloaded from NCBI and GISAID databases. If any two of the primers were mapped to a sequence on opposite strands with short distance apart, potential amplifications were flagged. No potential unintended cross reactivity is expected based on this *in silico* analysis.

Table 12 In silico analysis for SARS-CoV-2

Strain	*In Silico* Analysis for % Identity to Target 1 (nCoV)	*In Silico* Analysis for % Identity to Target 2 (Pan-Sarbecovirus 1)
CoV 229E	74.47	No alignment was found*
CoV OC43	72.26	No alignment was found*
CoV HKU1	76.52	No alignment was found*
CoV NL63	71.32	No alignment was found*
SARS-CoV	95.04	100
MERS	No alignment was found*	No alignment was found*
AdV	No alignment was found*	No alignment was found*
HMPV	No alignment was found*	No alignment was found*
HPIV1	No alignment was found*	No alignment was found*
HPIV2	No alignment was found*	No alignment was found*
HPIV3	No alignment was found*	No alignment was found*
HPIV4	No alignment was found*	No alignment was found*
Flu A	No alignment was found*	No alignment was found*
Flu B	No alignment was found*	No alignment was found*
EV	No alignment was found*	No alignment was found*
RSV	No alignment was found*	No alignment was found*
RV	No alignment was found*	No alignment was found*
Chlamydia pneumoniae	No alignment was found*	No alignment was found*

Strain	*In Silico* Analysis for % Identity to Target 1 (nCoV)	*In Silico* Analysis for % Identity to Target 2 (Pan-Sarbecovirus 1)
Haemophilus influenzae	No alignment was found*	No alignment was found*
Legionella pneumophila	No alignment was found*	No alignment was found*
MTB Mycobacterium bovis subsp. Bovis	No alignment was found*	No alignment was found*
Streptococcus pneumoniae	No alignment was found*	No alignment was found*
Streptococcus pyrogenes	No alignment was found*	No alignment was found*
Bordetella pertussis	No alignment was found*	No alignment was found*
Mycoplasma pneumoniae	No alignment was found*	No alignment was found*
Pneumocystis jirovecii	No alignment was found*	No alignment was found*
Influenza C	No alignment was found*	No alignment was found*
Parechovirus	No alignment was found*	No alignment was found*
Candida albicans	No alignment was found*	No alignment was found*
Corynebacterium diphtheriae	No alignment was found*	No alignment was found*
Legionella non-pneumophila	No alignment was found*	No alignment was found*
Bacillus anthracosis (Anthrax)	No alignment was found*	No alignment was found*
Moraxella cararrhalis	No alignment was found*	No alignment was found*
Neisseria elongate and meningitides	No alignment was found*	No alignment was found*
Pseudomonas aeruginosa	No alignment was found*	No alignment was found*
Staphylococcus epidermis	No alignment was found*	No alignment was found*
Staphylococcus salivarius	No alignment was found*	No alignment was found*
Leptospirosis	No alignment was found*	No alignment was found*
Chlamydia psittaci	No alignment was found*	No alignment was found*
Coxiella burneti (Q-Fever)	No alignment was found*	No alignment was found*
Streptococcus aureus	No alignment was found*	No alignment was found*

Note: * The amplicon sequences were blasted against all the exclusive sequences with very low stringency cutoff (50% and 100bp). No alignment were found passing the cutoff and no concerns for cross-reactivity were observed.

Cross reactivity testing

Cross-reactivity of **cobas**® SARS-CoV-2 was evaluated by testing whole organisms. As listed in Table 13, a panel of multiple unique sub-species of microorganisms were tested. High titer stocks of the potentially cross-reacting microorganisms were spiked into negative simulated clinical matrix to a concentration level of 1.0E+05 units/mL for viruses and 1.0E+06 units/mL for other microorganisms, unless otherwise noted.

None of the organisms tested interfered with **cobas**® SARS-CoV-2 performance by generating false positive results.

Table 13 Cross-reactivity test results

Microorganism	Concentration	Target 1 Result	Target 2 Result
Human coronavirus 229E	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Human coronavirus OC43	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Human coronavirus HKU1	1.0E+05 cp/mL	Negative	Negative
Human coronavirus NL63	1.0E+05 $TCID_{50}$/mL	Negative	Negative
MERS coronavirus	1.0E+05 genomic equivalent/mL	Negative	Negative
SARS coronavirus	1.0E+05 PFU/mL	Negative	Positive
Adenovirus B (Type 34)	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Human Metapneumovirus (hMPV)	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Parainfluenza virus Type 1	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Parainfluenza virus Type 2	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Parainfluenza virus Type 3	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Parainfluenza virus Type 4	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Influenza A (H1N1)	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Influenza B	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Enterovirus E (Type 1)	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Respiratory syncytial virus	1.0E+05 PFU/mL	Negative	Negative
Rhinovirus	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Chlamydia pneumonia	1.0E+06 $TCID_{50}$/mL	Negative	Negative
Haemophilus influenzae	1.0E+06 CFU/mL	Negative	Negative
Legionella pneumophila	1.0E+06 CFU/mL	Negative	Negative
Mycobacterium tuberculosis	1.0E+06 cells/mL	Negative	Negative
Streptococcus pneumonia	1.0E+06 CFU/mL	Negative	Negative
Streptococcus pyrogenes	1.0E+06 CFU/mL	Negative	Negative
Bordetella pertussis	1.0E+06 CFU/mL	Negative	Negative
Mycoplasma pneumoniae	1.0E+06 CFU/mL	Negative	Negative
Pooled human nasal wash	5 - 50%	Negative	Negative

Sample type equivalency

Equivalence between nasopharyngeal swab (NPS) and oropharyngeal swab (OPS) sample types was evaluated using cultured virus (USA-WA1/2020 strain) spiked into paired negative samples (individual samples, not pooled) to prepare contrived low positive (approximately 1.5x Target 1 LoD) and moderate positive (approximately 4x Target 1 LoD) samples for each sample type. A total of 21 low positive paired samples, 11 moderate positive paired samples, and 11 negative paired samples were tested.

As shown in Table14, all low positive and moderate positive paired samples were positive in both sample matrices. All negative paired samples were negative in both sample types. The observed Ct values for contrived positive samples were comparable in both sample types.

Table14 Nasopharyngeal vs oropharyngeal sample type comparison

Sample Type	Sample Concentration	N	Target 1		Target 2	
			% Positive	Mean Ct (95% CI)	% Positive	Mean Ct (95% CI)
NPS	~1.5x LoD (Target 1)	21	100	31.9 (31.7 – 32.0)	100	33.6 (33.5 – 33.7)
OPS			100	32.2 (31.8 – 32.6)	100	33.7 (33.4 – 34.1)
NPS	~4x LoD (Target 1)	11	100	30.9 (30.3 – 31.5)	100	32.2 (31.6 – 32.9)
OPS			100	31.5 (31.2 – 31.9)	100	32.7 (32.4 – 33.0)
NPS	Negative	11	0	n/a	0	n/a
OPS			0	n/a	0	n/a

Clinical evaluation

The performance of **cobas**® SARS-CoV-2 with prospectively collected nasopharyngeal swab clinical samples was evaluated using 100 individual negative clinical samples and 50 contrived positive clinical samples collected from patients with signs and symptoms of an upper respiratory infection.

Clinical samples were collected by qualified personnel according to the package insert of the collection device. Samples were handled as described in the package insert of the collection device and stored frozen until use. Samples were tested to be negative by a commercially available nucleic acid test for the qualitative detection of microorganisms associated with common upper respiratory tract infections.

Low positive and moderate positive contrived positive clinical samples were prepared by spiking cultured virus (USA-WA1/2020 strain) into individual negative clinical samples to approximately ~1.5x LoD (Target 1) (25 samples) and ~4x LoD (Target 1) (25 samples), respectively.

As shown in Table15 all low positive and moderate positive samples were positive and all negative samples were negative in the background of individual clinical sample matrix.

Table15 Clinical evaluation with nasopharyngeal swab samples

Sample Concentration	N	Target 1		Target 2	
		% positive (two-sided 95% CI)	Mean Ct	% positive (two-sided 95% CI	Mean Ct
~1.5x LoD	25	100 (86.7 – 100)	31.6	100 (89 – 100)	33.2
~4x LoD	25	100 (86.7 – 100)	31.1	100 (89 – 100)	32.4
Negative	100	0 (n/a)	n/a	0 (n/a)	n/a

Performance against the expected results are:
Positive Percent Agreement 50/50 = 100% (95% CI: 86.7% - 100%)
Negative Percent Agreement 100/100 = 100% (95% CI: 96.3% - 100%)

Conditions of Authorization for the Laboratory

The cobas SARS-CoV-2 test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website: https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm

Use of the cobas SARS-CoV-2 test must follow the procedures outlined in these manufacturer's Instructions for Use and the conditions of authorization outlined in the Letter of Authorization. Deviations from the procedures outlined are not permitted under the Emergency Use Authorization (EUA). To assist clinical laboratories running the cobas SARS-CoV-2 test, the relevant Conditions of Authorization are listed verbatim below, and are required to be met by laboratories performing the EUA test.

A. Authorized laboratories[1] using the cobas SARS-CoV-2 test will include with result reports of the cobas SARS-CoV-2 test, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
B. Authorized laboratories using the cobas SARS-CoV-2 test will perform the cobas SARS-CoV-2 test as outlined in the cobas SARS-CoV-2 Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the cobas SARS-CoV-2 test are not permitted.
C. Authorized laboratories that receive the cobas SARS-CoV-2 test must notify the relevant public health authorities of their intent to run the test prior to initiating testing.
D. Authorized laboratories using the cobas SARS-CoV-2 test will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
E. Authorized laboratories will collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Roche Diagnostics US Customer Technical Support 1-800-526-1247 any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
F. All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
G. RMS, its authorized distributor(s) and authorized laboratories using the cobas SARS-CoV-2 test will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] For ease of reference, this letter will refer to, "United States (U. S.) laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate complexity tests, and in U.S. laboratories certified under CLIA to perform high complexity tests" as "authorized laboratories."

Additional information

Key test features

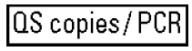
Sample type	Nasopharyngeal and oropharyngeal swab samples collected in the Copan UTM-RT System or the BD™ UVT System
Minimum amount of sample required	0.6 mL*
Sample processing volume	0.4 mL
Test duration	Results are available within less than 3.5 hours after loading the sample on the system.

*Dead volume of 0.2 mL is identified for the **cobas omni** Secondary tubes. Other tubes compatible with **cobas**® 6800/8800 Systems (consult User Assistance Guide) may have different dead volume and require more or less minimum volume.

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 16 Symbols used in labeling for Roche PCR diagnostics products

	Ancillary Software		Lower Limit of Assigned Range		Negative Control
	Authorized representative in the European community		Upper Limit of Assigned Range		Positive Control
	Barcode Data Sheet		Store in the dark		Control
	Batch code		Contains sufficient for <*n*> tests		Assigned Range (copies/mL)
	Biological risks		Temperature limit		Assigned Range (IU/mL)
	Catalogue number		Test Definition File		Standard Procedure
	Consult instructions for use		Manufacturer		Ultrasensitive Procedure
	Contents of kit		Use-by date		QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.
	Distributed by		Global Trade Item Number		QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
	For IVD performance evaluation only		Serial number		This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.
	US Only: Federal law restricts this device to sale by or on the order of a physician.		Date of manufacture		
	*In Vitro* diagnostic medical device		Do not reuse		

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 17 Manufacturer and distributors

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876 USA
www.roche.com

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the
Roche Response Center
toll-free: 1-800-526-1247)

Trademarks and patents

See http://www.roche-diagnostics.us/patents

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References

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2. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.

Document revision

Document Revision Information	
Doc Rev. 1.0 03/2020	First Publishing.